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Human Cytomegalovirus Glycoprotein B Genotypes in Blood of AIDS Patients: Lack of Association With Either the Viral DNA Load in Leukocytes or Presence of Retinitis

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It has been suggested that human cytomegalovirus (HCMV) glycoprotein B (gB) genotypes could be used as a marker for viral virulence in patients with AIDS. The present study was designed to evaluate a possible association between specific gB genotypes, the presence of HCMV retinitis, and the HCMV viral load. Fifty-four blood samples were obtained from 54 HIV- and HCMV-infected patients. Twenty-seven of these patients were asymptomatic for HCMV, whereas the other 27 patients had been diagnosed recently with HCMV retinitis. HCMV gB genotyping was carried out by using restriction enzyme analysis of PCR-amplified PMNL extracts. Determination of the HCMV viral load in the same specimens was carried out using a quantitative-PCR. HCMV gB genotype 2 was found more frequently than other genotypes in PCR-amplified polymorphonuclear leukocytes (PMNL) of patients with AIDS ($P < 0.05$) but not more frequently in samples from patients with HCMV retinitis. No significant association was found between any HCMV gB genotypes and the viral load in blood. In conclusion, the actual HCMV gB genotyping system using PMNL provides no additional benefit over the viral load in blood for identification of HIV-infected subjects at risk of HCMV disease. ***J. Med. Virol. 59:98–103, 1999.***

KEY WORDS: human cytomegalovirus; gB genotypes; risk for HCMV retinitis; viral load

INTRODUCTION

Human cytomegalovirus (HCMV), a member of the betaherpesvirinae subfamily, causes significant morbidity and mortality among immunocompromised patients. In these patients, the degree of immunosuppression correlates generally with the severity of HCMV infection. Moreover, the nature of the immunosuppression appears to modulate the type of HCMV disease in terms of affected organs [van der Meer et al., 1996]. Interestingly, for similar degree of immunosuppression and even for comparable systemic viral load, some patients will develop HCMV disease while others will remain asymptomatic. Possible explanations for this observation include host susceptibility factors as well as poorly described viral virulence factors resulting in selected tropism of some HCMV strains.

An abundant component of the virion envelope, the glycoprotein B (gB), is known to be a key target for both antibody- and cell-mediated immune responses [Cranage et al., 1986; Hopkins et al., 1996]. This glycoprotein is implicated in virion penetration and cell-to-cell viral transmission [Navarro et al., 1993]. Sequence analysis of the HCMV gB gene has revealed limited variation among clinical isolates from various geographical locations. Based on their gB peptide configurations, Chou and Dennison [1991] have classified HCMV isolates into one of four genotypes. Given its role in viral infectivity, the gB molecule has been considered as a potential marker for viral virulence.

Some studies have attempted to correlate HCMV gB genotypes with clinical outcome for different populations of immunocompromised subjects. Whether or not particular gB genotypes are associated with a greater risk of developing HCMV disease due to a specific tissue tropism remains unclear [Bongarts et al., 1996; Fries et al., 1994; Meyer-König et al., 1998b; Peek et

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al., 1998; Rasmussen et al., 1997; Shepp et al., 1996; Torok-Storb et al., 1997; Vogelberg et al., 1996; Woo et al., 1997; Zipeto et al., 1998]. Since the viral load is known as an important risk factor for HCMV disease in patients with AIDS [Boivin et al., 1997; Bowen et al., 1997; Dodt et al., 1997; Rasmussen et al., 1995; Shinkai et al., 1997], some genotypes could also predispose to HCMV disease by a different mechanism e.g. by facilitating viral replication and increasing the systemic viral load.

In the present study, the HCMV gB genotypes of PCR-amplified polymorphonuclear leukocyte (PMNL) extracts from AIDS patients with or without HCMV retinitis was characterized. The HCMV gB genotypes in PMNL were then correlated with the HCMV DNA load in the same samples, as well as with the presence or absence of HCMV retinitis.

MATERIALS AND METHODS

Samples and Viral Load

Samples collected for this study were recovered from HIV- and HCMV-seropositive subjects (> 80% were homosexual white males) with CD4 T-cell counts < 100/mm^3 enrolled in a prospective HCMV viremia study [Boivin et al., 1997]. A blood sample was tested for gB genotyping from all asymptomatic subjects and from those with typical HCMV retinitis within 24 hours prior to onset of antiviral therapy. Sequential blood samples were also tested over time for a subset of patients who developed HCMV disease. PMNL were obtained from EDTA-treated blood samples after centrifugation and standard dextran sedimentation procedure. DNA was extracted using a rapid cell lysis protocol and the viral load was quantified as described previously [Boivin et al., 1997]. Briefly, a part of the HCMV Major Immediate Early (MIE) gene was amplified from both patients' PMNL extracts and serial dilutions of a plasmid DNA containing the MIE gene. Detection of PCR products was achieved using a nonisotopic hybridization assay (SHARP Signal System; Digene Diagnostics Inc., Silver Spring, MD). A standard curve was then constructed by plotting optical density (OD) values obtained for the different dilutions of the plasmid against the number of input plasmid copies. Patients' viral load in PMNL was obtained by plotting OD values of the specimens into the standard curve. The HCMV viral load was reported as the number of HCMV copies per 10^5 PMNL.

gB Genotyping

The HCMV gB genotype was determined directly from patients' PMNL using nested PCR and restriction enzyme analysis based on the classification scheme proposed by Chou [1990] and Chou and Dennison [1991]. Briefly, a part of the HCMV gB gene was amplified with primers gB1043/1724 (codons 348-575) using DNA extracted from 2×10^4 PMNL. A second round of PCR was undertaken with internal primers gB1319/1604 (codons 440-535) and 2 μl of the first PCR. Amplified DNA samples were divided into two aliquots (10 μl each) for digestion with RsaI and HinfI [Chou, 1990]. Restriction patterns were visualized after electrophoresis on an 8% polyacrylamide gel stained with ethidium bromide. Specimens were classified into one of the four gB genotypes according to their specific enzymatic restriction patterns [Chou and Dennison, 1991]. A gB infection was defined by the presence of a gB genotype (alone or within a mixed population) in PMNL samples, whereas a predominant gB infection was defined by a unique or predominant genotype as assessed visually on the gel.

Statistical Analyses

Statistical analyses to evaluate the association between HCMV gB genotypes and HCMV viral load as well as between the presence of retinitis and HCMV viral load were carried out using the Wilcoxon rank-sum test. A Fisher's exact test was used to assess the association between gB genotypes and viral load as a dichotomized variable and between gB genotypes and presence of retinitis. A McNemar's (exact) test was used to assess the distribution of gB genotypes in PMNL of patients. A multivariate logistic regression model was also used to verify the relevance of specific gB genotypes by adjusting for the viral load. In this model, the response variable was the patients' outcome (retinitis or not), while independent variables included in the model were the genotypes and the viral load (dichotomized as < 1,000 or ≥ 1,000 HCMV copies per 10^5 PMNL).

RESULTS

Distribution of gB Genotypes in PMNL

HCMV DNA load and gB genotypes were determined in 54 PMNL extracts from 54 HIV- and HCMV-infected patients. Twenty-seven of these patients were asymptomatic at the time of the analysis and remained retinitis-free for a mean follow-up of 295.7 days (median: 169.0; range: 0–1 020 days), whereas 27 other patients have been recently diagnosed with HCMV retinitis. For the latter subjects, only baseline (pre-therapy) specimens were analyzed. HCMV gB genotypes 1, 2, 3, and 4 were detected in 16 (29.6%); 31 (57.4%); 9 (16.6%), and 18 (33.3%) of the 54 specimens, respectively. HCMV gB genotype 2 was detected more frequently than genotypes 1 ($P = 0.032$), 3 ($P = 0.0001$), and 4 ($P = 0.047$). Mixtures of HCMV gB genotypes were present in 20 (37.0%) of the 54 specimens examined. Sequential specimens (mean, 5.7; range, 3 to 8) were also examined in six patients who developed eventually HCMV disease (mean period of follow-up, 343.3 days; range, 26 to 651). HCMV gB genotypes and HCMV DNA load detected over time in PMNL of these six patients are summarized in Figure 1. For three of the patients (1, 3, and 4), one single gB genotype was detected over time. In two patients (patients 5 and 6), a single genotype was detected in all PMNL specimens throughout follow-up with an additional genotype occasionally detected. Finally, in the last patient (patient 2), a genotype (gB 4) was detected in all specimens

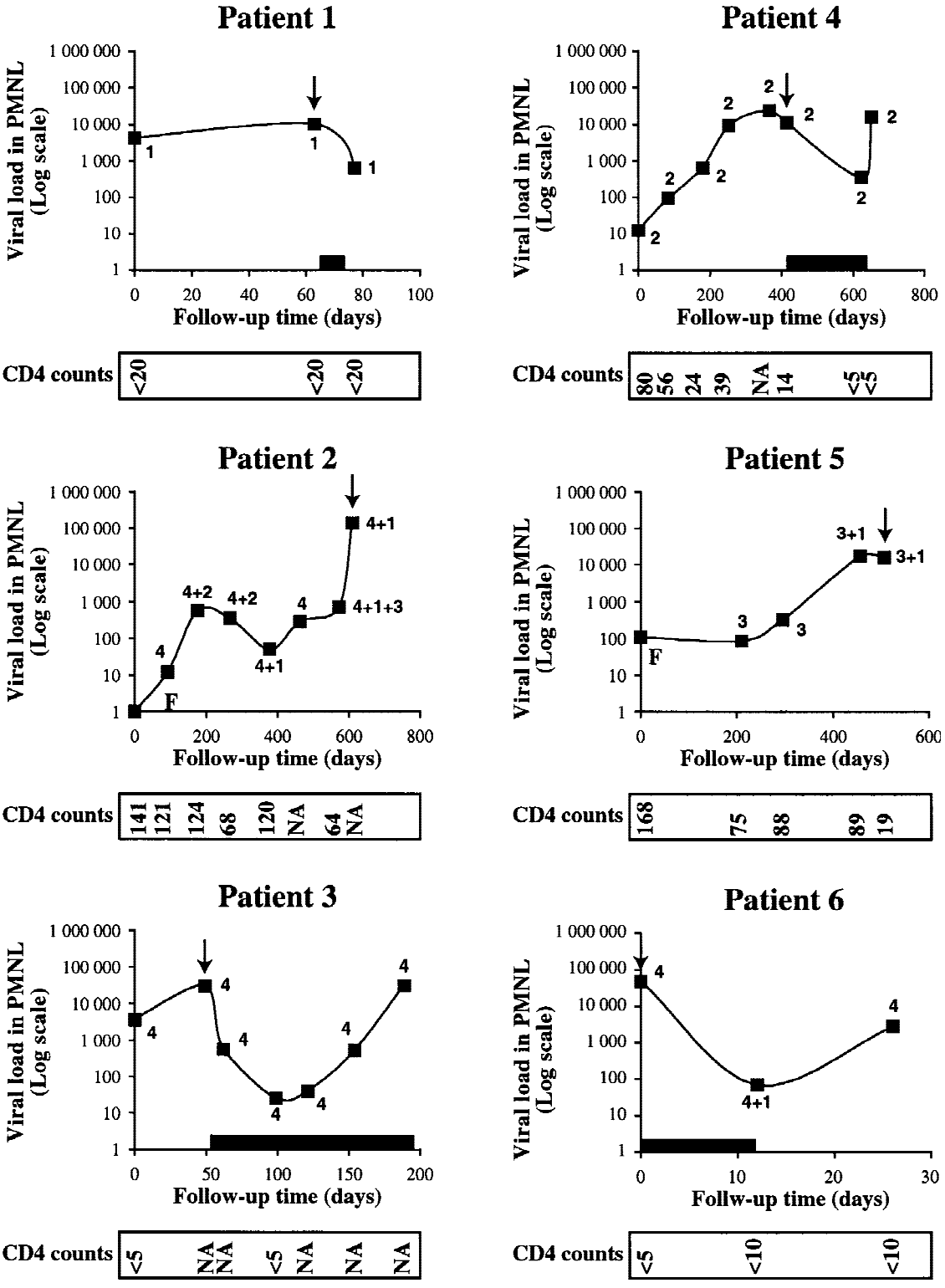

Fig. 1. Viral load and HCMV gB genotypes detected in sequential PMNL extracts from six patients with AIDS who developed HCMV diseases. Viral load in PMNL is expressed as the number of HCMV DNA copies per 10^5 PMNL. Numbers surrounding the curves represent gB genotypes detected in sequential samples. Arrows indicate the time at which diagnosis of HCMV disease was made. Horizontal filled bars represent intravenous courses of either ganciclovir or foscarnet. CD4 T-cell counts (in the boxes) are expressed as the number of CD4 cells per mm^3. NA indicates that CD4 counts were not available. F indicates that gB genotype could not be determined due to failure of amplification.

TABLE I. Relative Risk of a High Viral Load (≥1,000 copies) in PMNL by HCMV gB Genotypes

Detected genotype in PMNL	gB 1 Odds ratio (*P* value)	gB 2 Odds ratio (*P* value)	gB 3 Odds ratio (*P* value)	gB 4 Odds ratio (*P* value)
Present[a]	0.6 (0.43)	1.6 (0.44)	2.5 (0.29)	1.6 (0.59)
Predominant/unique[a]	0.6 (0.54)	1.1 (1.00)	1.1 (1.00)	1.1 (1.00)

[a]Reference is the absence of the indicated genotype; Fisher's exact test was used.

tested but the three other genotypes were also present at different times during the study period.

Relationships Between gB Genotypes, Viral Load, And Outcome

The relationship between the systemic viral load and the development of HCMV retinitis was studied first. The mean viral load in PMNL of asymptomatic patients was 975.2 (median 261.0, range: 12.5 to 7 160) HCMV copies per 10^5 PMNL compared to 18 377.2 (median 7 067.0, range: 12.5 to 119 350) copies for patients with HCMV retinitis ($P = 0.0003$). A possible association between specific HCMV gB genotypes and the blood viral load was then studied by considering the latter variable as a continuous or a dichotomized (< 1,000 and ≥ 1,000 HCMV copies) variable. However, no significant relationship was found between a particular gB genotype (present or predominant) and the blood viral load as assessed as a continuous variable (*P* value > 0.10 in all cases using the Wilcoxon rank-sum test) or a dichotomized (< 1,000 and ≥ 1,000 copies) variable (Table I). Similarly, no significant association was found between any HCMV gB genotypes and presence of HCMV retinitis when studied in an univariate model (Table II). Because of the previous association between the HCMV viral load in PMNL and the presence of retinitis, a logistic regression model was constructed to adjust for the viral load (< 1,000 vs. ≥ 1,000 HCMV copies per 10^5 PMNL). Again, no gB genotype (either present or predominant) was significantly associated with HCMV retinitis (Table II).

DISCUSSION

In the present study, the interactions between HCMV gB genotypes, HCMV viral load in PMNL and presence of HCMV retinitis were evaluated in AIDS patients with low CD4 T-cell counts (< 100/mm^3). The only significant association was found between the HCMV DNA load in the blood and the presence of HCMV retinitis as previously reported by us and others [Rasmussen et al., 1995; Boivin et al., 1997; Bowen et al., 1997; Dodt et al., 1997; Shinkai et al., 1997]. Thus, specific HCMV gB genotypes as defined by actual methods [Chou and Dennison, 1991] do not seem to have an influence on the viral load or the development of retinitis in that population.

Recently, some studies reported a potential influence of some HCMV gB genotypes on clinical outcome in immunocompromised patients. Shepp et al. [1996] reported that presence of gB 2 in blood isolates was potentially associated with a greater risk of developing HCMV retinitis in patients with AIDS. The same association was also described in bone marrow transplant recipients (BMT) with HCMV disease [Woo et al., 1997]. In addition, Bongarts et al. [1996] reported that HCMV gB genotype 1 was encountered less frequently in patients with AIDS and HCMV retinitis. The latter study was in agreement with results obtained by Fries et al. [1994] who showed that infection with gB 1 was associated with a better clinical outcome after BMT. In contrast, other studies reported no association between HCMV gB genotypes and outcome in subjects with AIDS [Rasmussen et al., 1997; Peek et al., 1998; Zipeto et al., 1998] and in kidney transplant recipients [Vogelberg et al., 1996; Woo et al., 1997]. In agreement with the latter studies, our results indicate no association between specific HCMV gB genotypes and the presence of retinitis in subjects with AIDS. Even when the viral load was adjusted to control for this variable, no association could be found between any gB genotypes and the development of HCMV retinitis. As reported previously [Rasmussen et al., 1997; Zipeto et al., 1998], we found that patients with AIDS and low CD4 T-cell counts were infected more frequently with HCMV gB genotype 2 than with other genotypes $P < 0.05$). In fact, our study confirms the very high incidence of gB 2 among Canadian HIV-infected homosexual males (57.4% in this study) similar to other geographic areas (56% in California; 47% in Italy) [Zipeto et al., 1998].

Our study population was homogeneous (all patients had CD4 < 100/mm^3 and most were homosexual white males) and it is thus unlikely that immunologic or host factors biased the results of the analyses. Also, the use of clinical specimens (instead of viral isolates) decreased the possibility of a potential bias due to selection of strains that are more adapted to grow in a cell culture system. The latter point could explain the discrepancy between our findings and those obtained by other investigators who found a relationship between a specific gB genotype (gB 2) in viral isolates and development of HCMV retinitis [Shepp et al., 1996]. This hypothesis is further supported by the absence of mixed viral populations in their study. Another aspect to consider when comparing gB studies is the time of sample collection. In Shepp's study [Shepp et al., 1996], blood specimens were collected at different times with regard to the diagnosis of HCMV retinitis (up to 6 months prior or even after diagnosis). As observed in three of our six patients with sequential specimens

TABLE II. Relative Risk of HCMV Retinitis by HCMV gB Genotypes

Detected genotype in PMNL	gB 1 Odds ratio (*P* value)	gB 2 Odds ratio (*P* value)	gB 3 Odds ratio (*P* value)	gB 4 Odds ratio (*P* value)
Present[a]	0.7 (0.77)	1.6 (0.58)	1.3 (1.00)	2.0 (0.39)
Predominant/unique[a]	0.6 (0.73)	1.0 (1.00)	1.0 (1.00)	2.0 (0.50)
Present[b]	1.1 (0.91)	1.2 (0.80)	0.7 (0.62)	1.7 (0.42)
Predominant/unique[b]	1.0 (0.97)	0.9 (0.87)	0.8 (0.84)	2.1 (0.34)

[a]Reference group includes subjects with asymptomatic HCMV infections; no adjustment for viral DNA loads; Fisher's exact test was used.
[b]Reference is the absence of the indicated genotype; after adjustment (<1,000 vs ≥1,000 HCMV copies) for viral load using a logistic regression model.

analyzed (Fig. 1), gB genotypes present in the blood can sometimes vary within a period of 6 months.

Because of the strong association that exists between an increasing viral load in the blood and development of HCMV retinitis in patients with AIDS, it is tempting to speculate that the viral strain that is implicated in the retinal lesions is more likely to be present in the blood at the moment when the viral load is high. However, the possibility cannot be excluded that, in some of the patients, HCMV retinitis was caused by a genotype that would have reached the retina during a previous episode of asymptomatic HCMV viremia. In fact, there is some evidence that different gB populations may infect the eye and blood compartments at the same time [Peek et al., 1998; Verbraak et al., 1998]. However, a recent study revealed that the distribution of gB genotypes in the eyes of AIDS patients with HCMV retinitis closely resembles previously reported distribution of these genotypes in the blood of AIDS patients with low CD4 T-cell counts making unlikely that some genotypes possess selected ocular tropism [Chern et al., 1998].

The results do not exclude the possibility that specific gB genotypes could influence clinical outcome in other AIDS-related HCMV diseases such as encephalitis or in subjects other than AIDS patients, as reported for BMT recipients [Fries et al., 1994; Torok-Storb et al., 1997; Woo et al., 1997]. It should also be noted that the greatest odds ratio for HCMV retinitis in our study was obtained for gB genotype 4 (OR 2.1; $P = 0.34$). Thus, it is possible that specific gB associations might also exist in subjects with AIDS although a much larger study would be required to confirm or refute this hypothesis.

In conclusion, the data suggest that specific HCMV gB genotypes are probably not associated with a replicative advantage in PMNL of patients with AIDS as shown by their lack of association with the HCMV DNA load. The results also suggest, as reported by others [Peek et al., 1998; Rasmussen et al., 1997; Zipeto et al., 1998], that the actual HCMV glycoprotein B classification scheme cannot be used as a marker for predicting HCMV retinitis in HIV-infected subjects. In that regard, Meyer-König et al. [1998a] recently reported that the current classification scheme based on the analysis of a short fragment of DNA representing the area surrounding the cleavage site of the gB glycoprotein may not be representative of the biologic properties of the whole protein. Thus, it is still possible that some viral virulence factors (gB or others) could be important in the pathogenesis of HCMV disease. So far, however, the determination of the immunologic status (CD4 T-cell counts) and the circulating viral load are the best prognostic markers for HCMV retinitis in AIDS patients [Boivin et al., 1997; Gallant et al., 1992; Rasmussen et al., 1995].

Consent

Informed consent was obtained from all patients enrolled in this study in accordance with guidelines in use at Université Laval, Université de Montréal and McGill University.

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REFERENCES

Boivin G, Handfield J, Murray G, Toma E, Lalonde R, Lazar JG, Bergeron MG. 1997. Quantitation of cytomegalovirus (CMV) DNA in leukocytes of human immunodeficiency virus-infected subjects with and without CMV disease by using PCR and the SHARP Signal Detection System. J Clin Microbiol 35:525–526.

Bongarts A, von Laer D, Vogelberg C, Ebert K, van Lunzen J, Garweg J, Vaith P, Hufert FT, Haller O, Meyer-König U. 1996. Glycoprotein B genotype of human cytomegalovirus: distribution in HIV-infected patients. Scand J Infect Dis 28:447–449.

Bowen EF, Sabin CA, Wilson P, Griffiths PD, Davey CC, Johnson MA, Emery VC. 1997. Cytomegalovirus (CMV) viraemia detected by polymerase chain reaction identifies a group of HIV-positive patients at high risk of CMV disease. AIDS 11:889–893.

Chern KC, Chandler DB, Martin DF, Kuppermann BD, Wolitz RA, Margolis TP. 1998. Glycoprotein B subtyping of cytomegalovirus (CMV) in the vitreous of patients with AIDS and CMV retinitis. J Infect Dis 178:1149–1153.

Chou S. 1990. Differentiation of cytomegalovirus strains by restriction analysis of DNA sequences amplified from clinical specimens. J Infect Dis 162:738–742.

Chou S, Dennison KM. 1991. Analysis of interstrain variation in cytomegalovirus glycoprotein B sequences encoding neutralization-related epitopes. J Infect Dis 163:1229–1234.

Cranage MP, Kouzarides T, Bankier AT, Satchwell S, Weston K, Tomlinson P, Barrell B, Hart H, Bell SE, Minson AC, Smith GL. 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J 5:3057–3063.

Dodt KK, Jacobsen PH, Hofmann B, Meyer C, Kolmos HJ, Skinhoj P, Norrild B, Mathiesen L. 1997. Development of cytomegalovirus (CMV) disease may be predicted in HIV-infected patients by CMV

polymerase chain reaction and the antigenemia test. AIDS 11:F21–F28.

Fries BC, Chou S, Boeckh M, Torok-Storb B. 1994. frequency distribution of cytomegalovirus envelope glycoprotein genotypes in bone marrow transplant recipients. J Infect Dis 169:769–774.

Gallant JE, Moore RD, Richman DD, Keruly J, Chaisson RE. 1992. Incidence and natural history of cytomegalovirus disease in patients with advanced human immunodeficiency virus disease treated with zidovudine: The Zidovudine Epidemiology Study Group. J Infect Dis 166:1223–1227.

Hopkins JI, Fiander AN, Evans AS, Delchambre M, Gheysen D, Borysiewicz LK. 1996. Cytotoxic T cell immunity to human cytomegalovirus glycoprotein B. J Med Virol 49:124–131.

Meyer-König U, Haberland M, von Laer D, Haller O, Hufert FT. 1998a. Intragenic variability of human cytomegalovirus glycoprotein B in clinical strains. J Infect Dis 177:1162–1169.

Meyer-König U, Vogelberg C, Bongarts A, Kampa D, Delbruck R, Wolff-Vorbeck G, Kirste G, Haberland M, Hufert FT, von Laer D. 1998b. Glycoprotein B genotype correlates with cell tropism in vivo of human cytomegalovirus infection. J Med Virol 55:75–81.

Navarro D, Paz P, Tugizov S, Topp K, La Vail J, Pereira L. 1993. Glycoprotein B of human cytomegalovirus promotes virion penetration into cells, transmission of infection from cell to cell, and fusion of infected cells. Virology 197:143–158.

Peek R, Verbraak V, Bruinenberg M, van der Lelij A, van den Horn G, Kijlstra A. 1998. Cytomegalovirus glycoprotein B genotyping in ocular fluids and blood of AIDS patients with cytomegalovirus retinitis. Invest Ophthalmol Visual Sci 39:1183–1187.

Rasmussen L, Morris S, Zipeto D, Fessel J, Wolitz R, Dowling A, Merigan TC. 1995. Quantitation of human cytomegalovirus DNA from peripheral blood cells of human immunodeficiency virus-infected patients could predict cytomegalovirus retinitis. J Infect Dis 171:177–182.

Rasmussen L, Hong C, Zipeto D, Morris S, Sherman D, Chou S, Miner R, Drew WL, Wolitz R, Dowling A, Warford A, Merigan TC. 1997. Cytomegalovirus GB genotype distribution differs in human immunodeficiency virus-infected patients and immunocompromised allograft recipients. J Infect Dis 175:179–184.

Shepp DH, Match ME, Ashraf AB, Lipson SM, Millan C, Pergolizzi R. 1996. Cytomegalovirus glycoprotein B groups associated with retinitis in AIDS. J Infect Dis 174:184–187.

Shinkai M, Bozzette SA, Powderly W, Frame P, Spector SA. 1997. Utility of urine and leukocyte cultures and plasma DNA polymerase chain reaction for identification of AIDS patients at risk for developing human cytomegalovirus disease. J Infect Dis 175:302–308.

Torok-Storb B, Boeckh M, Hoy C, Leisenring W, Myerson D, Gooley T. 1997. Association of specific cytomegalovirus genotypes with death from myelosuppression after marrow transplantation. Blood 90:2097–2102.

van der Meer JT, Drew WL, Bowden RA, Galasso GJ, Griffiths PD, Jabs DA, Katlama C, Spector SA, Whitley RJ. 1996. Summary of the international consensus symposium on advances in the diagnosis, treatment and prophylaxis of cytomegalovirus infection. Antiviral Res 32:119–140.

Verbraak V, Bruinenberg M, van den Horn G, Meenken C, van der Lelij A, Hoyng CB, Kijlstra A, Peek R. 1998. Cytomegalovirus (CMV) strain differences between the eye and blood in AIDS patients with CMV retinitis. AIDS 12:713–718.

Vogelberg C, Meyer-König U, Hufert FT, Kirste G, von Laer D. 1996. Human cytomegalovirus glycoprotein B genotypes in renal transplant recipients. J Med Virol 50:31–34.

Woo PC, Lo CY, Lo SK, Siau H, Peiris JS, Wong SS, Luk WK, Chan TM, Lim WW, Yuen KY. 1997. Distinct genotypic distributions of cytomegalovirus (CMV) envelope glycoprotein in bone marrow and renal transplant recipients with CMV disease. Clin Diag Lab Immunol 4:515–518.

Zipeto D, Hong C, Gerna G, Zavattoni M, Katzenstein D, Merigan TC, Rasmussen L. 1998. Geographic and demographic differences in the frequency of human cytomegalovirus GB genotypes 1-4 in immunocompromised patients. AIDS Res Hum Retroviruses 14:533–536.